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SOME USES OF STATISTICS IN PLANT PATHOLOGY

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The plant pathologist is concerned with the study and control of plant diseases, including those caused by fungi, bacteria, and those of virus origin. In such investigations, the complexity of interrelated variables makes it obvious that statistical methods should be of value in planning experiments and in interpreting results.

One of the important problems with which the phytopathologist must deal is the comparison of different chemical treatments for the control of plant disease in the laboratory, greenhouse, and in the field. Preliminary tests of fungicides are often made by germinating fungus spores in drops placed on glass slides, which have previously received a deposit of the fungicide, and which are incubated for a definite period of time. Results are usually expressed as percentage of spores failing to germinate at increasing dosages of applied fungicide after correcting for spores failing to germinate in the untreated control. A regression line may be fitted to the data after conversion of percentages to probits, and dosages to logarithms (7). It has been pointed out recently (15) that where there are appreciable numbers of spores failing to germinate in the controls the proper weights to attach to the probit values differ from those commonly used, and tables have been published giving weight factors for this purpose.

In a study of 600 such dosage effect curves (24), with various fungicides and several species of fungus spores it has been found that the slopes of the regression lines obtained vary widely with different fungicides, and furthermore the regression in many cases is not linear when probit values are plotted against the logarithm of the dose. have even been reported (12) in which a reversal of trend is encountered with increasing concentration of fungicide. When a group of fungicides is compared several times in successive experiments, there is a significant interaction of LD50 values with experiments (24). For this reason it has been considered advisable to estimate LD50 values graphically and to repeat comparisons on several different occasions, using the fungicide experiment interaction variance as the proper error term for comparing fungicides.

The fact that the slopes of dosage effect curves vary widely for different fungicides has led some investigators to attempt to draw conclusions regarding the mechanism of fungicidal action from the slopes and curvature of the regression line obtained. By a combination of statistical ideas with the equations of reaction kinetics, conclusions have been drawn as to the nature of the active fungicidal agent in a number of cases (26).

While spore germination tests on glass slides may be useful in a preliminary examination of possible fungicides, it is necessary to determine their behavior when applied to growing plants infected with pathogenic fungi. Greenhouse methods have been worked out

using artificially infected tomato plants (22). The plants were sprayed with suspensions of fungus spores and the lesions produced on the leaves were counted after a certain time. The results on plants treated with a fungicide previous to inoculation were expressed as percent disease control by dividing the number of lesions on three leaves of the treated plants by those on a similar set of three leaves on the controls, subtracting from one and multiplying by one hundred. When such experiments were performed with increasing concentrations of fungicide applied previous to inoculation, a dosage effect curve was obtained. Percent disease control converted to probits plotted against the logarithm of fungicide concentration gave a linear relationship, thus facilitating the comparison of different fungicides. The proper weights to attach to the observations differ from those used in the glass slide spore germination tests and were determined empirically from the observed variances. The maximum reproducibility was found at about 90 percent disease control. When experiments were repeated several times, a significant interaction between experiments and treatments was found. For this reason it was felt necessary to repeat comparisons at several different times, using the interaction as

In laboratories performing daily routine tests knowledge of the experimental error of a given procedure may be accumulated over a period of time and this estimate of error will be more precise than that made from a single experiment (23), provided a state of "statistical control" has been achieved. The use of such accumulated knowledge is similar to the control chart procedure so much used in industry.

Since the ultimate object of laboratory testing of fungicides is to be able to predict performance in the field, it is important to know the degree of correlation existing between laboratory, greenhouse and field experiments. A critical examination has been made (31) of the correlation between laboratory spore germination tests and greenhouse tomato foliage disease tests. The correlation was as good as could be expected for the majority of treatments, but there were notable exceptions with certain chemical types. In the

case of copper fungicides, studies have been made of the correlation between laboratory "tenacity" or resistance to washing, and tenacity in the field on cherry leaves (25). While the correlation was high, it was concluded that laboratory determination of toxicity was more important than tenacity in predicting the field performance of the materials studied.

Fungicides are often applied to seeds in order to control seed-borne diseases and soil organisms. Experiments may be performed in seed germination cabinets, in the greenhouse, or plantings may be made in the field. The data collected may be in the form of percent emergence, or percentage of healthy plants, or some more complex index of disease may be calculated.

The question of experimental design is important in order to obtain the maximum information with a minimum expenditure of time and material. Where the number of treatments is not large, the well-known Latin square and randomized block arrangements are satisfactory. With large numbers of treatments, it is desirable to make use of some of the newer designs such as balanced incomplete blocks, lattice, and lattice square designs.

Several studies have been made of the relative efficiency of such designs as compared to randomized blocks (6, 28). Since in these designs the number of treatments in a block is less than the total number of treatments, special methods of analysis are required to disentangle the block and treatment effects. In the lattice and lattice square designs, however, the experiment may always be treated as though it consisted of larger complete blocks if desired, and therefore the efficiency can never be less than that of randomized complete blocks, and may be considerably greater.

In carrying out the analysis of variance, there are certain conditions which should be fulfilled if the best use is to be made of the information at hand (10). The variances in the different portions of the experiment should be homogeneous, that is, should be drawn from the same population of variances. Tests for homogeneity of variance have been described (3, 19).

If the variances are not homogeneous they

can often be made so by a suitable transformation of the data (9). The angular transformation is used for percentages derived from the binomial series (5); the square root transformation for data from the Poisson series (4); while the logarithmic transformation has been used where the standard deviations tend to be proportional to the means. A critical study has been made (30) of an experiment on cotton wilt in relation to varieties and nutrition. Various transformations were investigated, particularly as to their effects on the interactions.

A recent paper (1) presents a novel method of comparing two seed treatments when the results were obtained as percentages of emergence and the variances were not homogeneous. The material consisted of the results of pea seed treatments from a number of localities. If the results of two treatments which were equally effective were plotted, the points would tend to lie along a 45° line between the two axes. By a transformation, the axes were rotated through 45° making this line the new axis of abscissas. A regression line was then fitted to the points, weighting according to the variance in different arrays. and the significance of the regression was tested.

An important problem in connection with plant diseases of virus origin is the estimation of the potency of an unknown virus preparation as compared with a standard. Some virus diseases produce local lesions on leaves when the latter are treated with a virus preparation. These lesions may be counted and serve as a measure of the potency. Careful studies have been made (27) of the accuracy of this type of procedure in the case of several virus diseases. The "unknown" in this case was a known dilution of a standard, and it was therefore possible to test whether the deviations from the true value were of a nature that would be expected from the error variance of the experiments. by means of the x2 test. Several modifications of the experimental design were studied. The actual counts were transformed to logarithms, and by analysis of variance the contributions of the various factors such as leaves, pots, slope of dilution curves, difference of preparations, etc., were isolated, together with

the appropriate error terms. The log ratio of potencies, together with its standard error, was calculated, using a modification of formulas published previously (7). The general conclusion was that the method was satisfactory except in the case of alfalfa mosaic virus. In this case, the potency of the unknown was overestimated and the value of χ^a indicated abnormal fluctuations which could not be explained.

A type of sampling problem which often arises is represented by the following: Potato tubers are planted by some agencies in the greenhouse and in the South in order to determine as early as possible how much virus disease is present in the seed stocks. The question of the most economical sample size has been discussed by several investigators (14, 16). This subject, which is of great importance in the sampling and inspection of manufactured products in industry, has been dealt with in several publications (13, 19, 29). Concepts have been developed, such as "lot tolerance percent defective," "producer's risk," "consumer's risk," etc., which might be usefully applied to plant disease sampling problems. Charts have been published which enable one to dispense with the labor of summing the terms of the binomial and Poisson series.

The ability to measure the intensity of plant disease in the field is a necessary prerequisite to the evaluation of the effectiveness of control measures, and may aid in the study of the manner in which the disease is disseminated from a central source of infection. In the case of some diseases, such as the covered smut of oats, each plant can be classified as diseased or healthy. In other cases, actual measurement is impractical, and some method of estimating the intensity is essential. A system of grading has been worked out, based on the Weber-Fechner law relating visual acuity to the logarithm of intensity of the stimulus (21). A series of grade numbers in arithmetic progression corresponds to percentages of disease in geometric progression, running from 0 up to 50%, and 100 down to 50%. These grade numbers represent equal ability of the observer to distinguish differences in intensity throughout the scale.

Two problems which often have a bearing on the mode of dissemination of plant disease are (a) the spatial distribution of diseased plants in a field, and (b) the change in this distribution with time. Methods have been devised to test whether diseased plants in a field are distributed at random, whether infection tends to spread from one plant to its neighbor in a row, and whether the distribution of disease at a later period is related to that observed at a previous time (11).

In a study of the Dutch elm disease, it was found that the probability of infection at increasing distance from an infected tree showed a linear relationship when percent of infected trees (as probits) was plotted against the logarithm of the distance (32). Another investigation (8) deals with the survival of cotton seedlings, in field and greenhouse, under various conditions of attack by seed- and soilborne fungi. The seeds were planted, five in a hill, and the question at issue was whether the presence of one or more diseased seeds in a hill affected the emergence of the remaining seeds in the hill, or whether the seeds behaved as random samples following the binomial series. The method used was to compare visually the calculated and observed curves of emergence for various average percentages. In the case of infection with rhizoctonia, it was found that there was an excessive proportion of seedling failures in hills originally containing one or more diseased seedlings, indicating a spread of this disease from one seedling to another in the hill.

The white pine blister rust has as alternate host, current and gooseberry plants (Ribes spp.). Control of the disease is effected by eradication of the alternate host. For this reason, the estimation of the magnitude and type of distribution of Ribes is of importance. It has been found (18) that the distribution of Ribes did not follow the Poisson distribution, but was made up of an "infectious" distribution superimposed on a Poisson distribution. A study of this same disease along the time axis (17) has shown that the logistic curve, used in connection with studies on population growth, represents the spread of infection over a period of years very well. Departures from the logistic curve due to Ribes eradication measures, or to natural changes in the Ribes population, could be expressed by fitting additional constants to the linear form of the logistic curve, thus making the latter a parabola. A different method was used in an investigation of Alternaria blight of tomatoes (2). Here the increase in percentage defoliation was plotted on arithmetic probability paper, with time as the abscissa. Straight lines could be fitted to the data in this form also. It appears that the logistic curve and a cumulative probability curve may be used in similar situations. This is not surprising since they resemble each other rather closely.

Occasions may arise where a rapid approximate method of statistical treatment is useful in dealing with plant disease data. For example, in comparing methods of disease control, it may be satisfactory to rate them by assigning rank numbers in serial order instead of dealing with percentages or other actual measurements. Appropriate methods have been described (20) and have proved useful in spite of the loss of efficiency involved.

The variety of plant pathological problems involving measurement of some kind offer opportunities for the application of statistical methods in this field, no less important than those in other branches of the biological and physical sciences.

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Application for entry as second-class matter is pending. The Biometrics Bulletin is published six times a year—in February, April, June, August, October and December—by the American Statistical Association for its Biometrics Section. Editorial Office: 1603 K Street, N. W., Washington 6, D. C.

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A PROBIT SCALE FOR SLIDE RULES

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In studies of the frequency distribution of the length and width of fungal spores of different species, the writer has made extensive use of a graphic probit method developed originally for the analysis of toxicological experiments. The preparation of the graph involves the following steps: (1) measurement of the spore length or width either with an ocular micrometer or by applying a metric ruler to the projected image or the spore or to its photograph, (2) entry of the reading on a tally sheet to obtain a frequency distribution, (3) cumulation of the frequencies, (4) calculation of the percentage cumulated frequencies, and (5) conversion of percentages to probits by a table such as that given by Bliss. It occurred to the author that much time could be saved by combining steps (4) and (5) into one operation with a slide rule carrying a probit scale.

When determining cumulative percentages, several numbers can be divided by a single divisor with one or two settings of the moving scale. The two upper scales of a 25 cm. slide rule cover two logarithmic cycles and are usually identical. The fixed scale is labeled A and the movable scale B. The digit 1 in the center of scale B is set opposite the total number of individuals on scale A and the cumulative percentages on scale B are read opposite the cumulative frequencies on scale A for all observations within the range of the two overlapping scales. The slide is then moved to place 1 at one end of the scale B under the total number and the remaining percentages are read as before.

By adding an extra scale to the moving slide, probits instead of percentages can be read directly. The extra scale is graduated from 2.7 to 7.0 probits in the interval from 1.07 to 97.72 percent, covering both cycles of the B scale. The probits at the lower end of the scale, such as those for 1 to 20 percent, can be read with much greater precision than those at the other end above 80 percent. This is remedied by double numbering of the probit scale in both directions from 5 probits, so that 4 and 6, and 3 and 7 occur together. Then instead of reading the probit for 112/125, for example, that for 13/125 would be read instead on the reversed scale.

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¹ C. I. Bliss, Ann. Appl. Bio. 22: 134-167 (1935); 24, 815-852 (1937)

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OBSERVATIONS ON WARTIME BIOMETRICS

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New problems arising from war needs and acute shortages of standard materials have placed pressure on biological research agencies for immediate results. This has naturally been felt by biometricians. Before the war many biometricians concentrated their efforts on developing new statistical designs, refining sampling techniques, and applying involved tests, analyses and transformations, these activities were valuable is attested by the fact that they are being continued if not increased during the war; however, this preoccupation with advanced technical problems has brought statisticians and biometricians a measure of disrepute among certain "practical" biologists. A common attitude among some biologists has been that statistics may be of value for the more academic research problems but that field experiments are for the most part concerned with techniques which are not sufficiently refined to justify statistical treatment. On the other hand other biologists who attempt to use statistical methods lend weight to this attitude by subordinating the practical biological aspects of their research to statistical techniques. As a result the wartime pressure for immediate practical answers has oftentimes widened the breach between biometrician and experimental biologist.

It is of major importance that more emphasis be placed upon the purely practical aspects of biometric training and techniques. When answers must be obtained with a minimum expenditure of man-power and time, it is urgent that experimental designs and procedures be most efficient, and that the immediate end desired be kept clearly in mind at all times. Failure to plan carefully may result in unreliable results. The basic principles or logic of experimentation, which are usually taken for granted among biometricians, are often but poorly comprehended by the research Biometricians can furnish valbiologist. uable aid to the war effort by emphasizing these basic principles and relegating the mathematical refinements to the background except in those instances in which the situation clearly justifies the more involved techniques.

The following examples may illustrate the types of situations in which it is essential that the principles of sound experimentation be followed, even though complicated designs and analyses are impossible.

The selection of the most satisfactory sampling technique is of prime importance to insure representativeness even if the degree of accurancy required is not high. Moreover, simple statistical tests may increase the confidence of the experimenter in his techniques. Consider the case of the control of mosquito larvae by chemical means. Anopheline larvae are surface feeders that tend to congregate around emergent vegetation and floating debris, and so are generally found near the grassy margins of ponds. Few larvae will be found in the open centers of ponds. It is impossible to count all of the larvae in a given pond and so a sampling technique designed to furnish an estimate of the relative population of the pond or a portion thereof before and after treatment is indicated. One suggestion has been to construct an apparatus designed to collect all of the larvae in a square foot of the pond surface to a depth of two inches. The mean obtained from a series of such units taken from a rather uniform quadrant would be used to estimate the population density in that section before and after treatment in terms of larvae per square foot. The difficulty is that the larvae are not evenly distributed over the entire surface so that density in terms of larvae per square foot has little meaning except in very homogeneous quadrants. The number per lineal foot of "intersection line" (i. e. the line where the plants intersect the air-water interface) would have more meaning though it would be much more difficult to compute. Also any such mechanical sampler is apt to disturb the emergent vegetation ahead of it and thus, the more active larvae may be missed.

A second suggestion has been to set up stations, one yard square, selected on the basis of the representativeness of the vegetation contained. All of the larvae in this previously measured square yard are counted. The most serious problem would be making sure that no larvae escape from the station during the counting process. If this difficulty is overcome it is possible that the technique might be satisfactory.

A third method that appeals to the "practical" field entomologist because of its simplicity and rapidity is to use long-handled dippers to sample the larvae in what the experimenter, on the basis of previous experience, believes to be the most likely spots to find larvae. The density in terms of larvae per dip is used as a purely relative index to the population.

A cursory survey of these three alternatives might indicate that the square foot sampler is best for it permits a large number of stratified random samples and seems to be interpretable in terms of the total population of the quadrant. The second choice would doubtless be the square yard permanent station for it would give a clear picture of population fluctuations in these representative areas. The dipper method has little to commend it except that it is simple and fast and if employed with good judgment, should produce larger figures since it is sampling the "ideal" loci rather than the entire area. This is of particular value in ponds of low population Under pressure for rapid results with a minimum of man-power these considerations become of major importance and so the dipper technique was put to a simple statistical test. A typical pond was divided into three concentric zones and into three sectors that cut across all three zones. Three experienced operators counted the larvae in these plots in such a way that each operator counted one plot from each zone and one plot from each sector. An analysis of the variation found between counts indicated a greater variance between zones and between sectors than between operators. Accordingly it was concluded that this very rapid sampling technique would be satisfactory for the exploratory research being conducted, providing the operators were careful to gather representative data in rather constant proportions from the various strata present in any pond. The actual evaluation of the proper weight to be given to each stratum would be very difficult because the strata are so ill-defined. It was felt that weighting by the operators as they sampled was sufficiently accurate for the problem at hand. Biometricians are usually skeptical of any sampling technique which is as dependent upon the judgment of the operator as is the dipping technique. It is unquestionably true that a more mechanical or randomized system of sampling removes the socalled "human factor" which is often very biased. However, in many exploratory experiments prompt acceptance or rejection of a treatment is much more important than the accuracy with which small differences are detected. Thus the biometrician must not overlook the possibility of leaving in the "human factor" and merely checking its magnitude occasionally to make sure that it does not grow to exceed the limits demanded by the problem.

A similar problem arises in relation to the arrangement of plots. The traditional contention between systematic and random arrangements (or the compromise of restricted random arrangements) may not be finally settled but the practical biometrician can often come to a conclusion in a specific case by a careful review of the objectives of the experiment. An example is the case in which the residual effect of an insecticidal deposit in a house was being studied. Various loci on the treated walls and ceiling were being tested for effectiveness at stated intervals after treatment. The question was how to select them. Should they be selected at random at each interval with the selection at a given interval entirely independent of the selections at previous or subsequent intervals, or should they be selected previous to the first testing and retained throughout all subsequent testings? Should the locations be distributed entirely at random or only at random within each of three very distinct strata (ceiling, inside walls, and outside walls)? If the stratified arrangement is accepted should the strata be weighted or should the loci be equally divided between the strata?

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OBSERVATIONS ON WARTIME BIOMETRICS

WILLIAM M. UPHOLT Carter Memorial Laboratory

New problems arising from war needs and acute shortages of standard materials have placed pressure on biological research agencies for immediate results. This has naturally been felt by biometricians. Before the war many biometricians concentrated their efforts on developing new statistical designs, refining sampling techniques, and applying involved tests, analyses and transformations, these activities were valuable is attested by the fact that they are being continued if not increased during the war: however, this preoccupation with advanced technical problems has brought statisticians and biometricians a measure of disrepute among certain "practical" biologists. A common attitude among some biologists has been that statistics may be of value for the more academic research problems but that field experiments are for the most part concerned with techniques which are not sufficiently refined to justify statistical treatment. On the other hand other biologists who attempt to use statistical methods lend weight to this attitude by subordinating the practical biological aspects of their research to statistical techniques. As a result the wartime pressure for immediate practical answers has oftentimes widened the breach between biometrician and experimental biologist.

It is of major importance that more emphasis be placed upon the purely practical aspects of biometric training and techniques. When answers must be obtained with a minimum expenditure of man-power and time, it is urgent that experimental designs and procedures be most efficient, and that the immediate end desired be kept clearly in mind at all times. Failure to plan carefully may result in unreliable results. The basic principles or logic of experimentation, which are usually taken for granted among biometricians, are often but poorly comprehended by the research biologist. Biometricians can furnish valuable aid to the war effort by emphasizing these basic principles and relegating the mathematical refinements to the background except in those instances in which the situation clearly justifies the more involved techniques. The following examples may illustrate the types of situations in which it is essential that the principles of sound experimentation be followed, even though complicated designs and analyses are impossible.

The selection of the most satisfactory sampling technique is of prime importance to insure representativeness even if the degree of accurancy required is not high. Moreover, simple statistical tests may increase the confidence of the experimenter in his techniques. Consider the case of the control of mosquito larvae by chemical means. Anopheline larvae are surface feeders that tend to congregate around emergent vegetation and floating debris, and so are generally found near the grassy margins of ponds. Few larvae will be found in the open centers of ponds. It is impossible to count all of the larvae in a given pond and so a sampling technique designed to furnish an estimate of the relative population of the pond or a portion thereof before and after treatment is indicated. One suggestion has been to construct an apparatus designed to collect all of the larvae in a square foot of the pond surface to a depth of two inches. The mean obtained from a series of such units taken from a rather uniform quadrant would be used to estimate the population density in that section before and after treatment in terms of larvae per square foot. The difficulty is that the larvae are not evenly distributed over the entire surface so that density in terms of larvae per square foot has little meaning except in very homogeneous quadrants. The number per lineal foot of "intersection line" (i. e. the line where the plants intersect the air-water interface) would have more meaning though it would be much more difficult to compute. Also any such mechanical sampler is apt to disturb the emergent vegetation ahead of it and thus, the more active larvae may be missed.

A second suggestion has been to set up stations, one yard square, selected on the basis of the representativeness of the vegetation

contained. All of the larvae in this previously measured square yard are counted. The most serious problem would be making sure that no larvae escape from the station during the counting process. If this difficulty is overcome it is possible that the technique might be satisfactory.

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The decision was based upon a close scrutiny of the objectives of the experiment. If the objective were to determine the shape or slope (or both) of the curve relating mortality to age of deposit without regard to the exact

position of the mean curve for the given room then the spots once selected should be kept constant throughout the balance of the experiment. In this way n points would provide estimates of n distinct curves all of which are expected to have the same parameters as regards shape and slope but not necessarily as regards position, for each of the loci may have received slightly different original dosages of the insecticide. Moreover the arrangement of the loci should be at random only within the strata and each stratum should have an equal number so that any possible differences between strata in regard to weathering and hence in shape or slope of curve could be detected with equal accuracy.

If the objective of the experiment were to determine the mean effectiveness of the deposit for a specific room at a given time after application then the arrangement would be quite different, for the position of the curve would be fully as important as its shape or slope. In such a case each individual point on the mean curve would be best estimated by n random determinations, the locations of which were entirely independent of the positions used in previous or subsequent determinations. Any dependence of the arrangement of loci upon previous or subsequent determinations would be apt to introduce a systematic error into the position of the curve. Moreover if any stratification of the sampling were employed, the weight given to each stratum would have to be determined on the basis of its biological or economic importance.

Actually the objective of the experiment was to develop a procedure which could be used in the field to determine when the houses in a given area (all of which were sprayed at the same time) should be resprayed. Therefore it was important neither to determine the shape or slope of the curve nor to determine the actual position of the curve for a given room. All that was necessary was an index to mean effectiveness so that when the mean index number for the area falls below a predetermined level, all the houses should be re-sprayed. The determination of the critical level must be based upon a careful correlation between the index number and the actual mean effectiveness of the insecticidal residue in the rooms being tested. The procedure

for finding the index number can very well be independent of systematic errors, which are always present but often undetected, within the three distinct strata in the room. Therefore a purely systematic arrangement of loci with just enough flexibility to permit it to fit into all rooms, can be used safely. Moreover such a systematic arrangement will be most efficient for the systematic errors will be removed from the estimate of the error term and the standard deviation will be reduced in size accordingly. Thus a given number of loci arranged in a pre-determined and constant systematic pattern will determine the index number with a greater accuracy than any random arrangement of the same number of loci could do. Since any bias that is introduced by the systematic arrangement will be constant for all determinations including the original determination of the critical level, no pertinent information is lost. Of course the selection of the houses to be tested should be purely random within the area.

A problem often encountered by the biometrician is the determination of the size of sample to be employed. This decision must always be made in the light of very practical considerations. It is of course necessary to have some estimate of the standard deviation that can be expected. It is equally important to know how small a difference between means must be detected with a predetermined frequency as well as with a known accuracy. Unfortunately the details for calculating this power of a test to detect real differences at a given level of significance are remarkably scant in the literature readily available to field men.

An example may be found in a case in which it was desired to determine the effects of certain mosquito-larviciding treatments upon the plankton found in small fresh-water ponds. The preliminary estimates of the standard deviations to be expected were based upon seven samples taken from an untreated pond. Each sample was thoroughly mixed and six aliquot portions were observed, the number of each of several groups of organisms being recorded. Analyzing each group of organisms separately, a highly significant difference between samples was indicated by the F-test, using the variance within samples

as the error term. The implication was clearly that the samples were not truly random or that the populations sampled were not distributed at random. Discussion with the microbiologist failed to uncover any marked faults in the sampling technique pparently the plankton population of the areas sampled was not homogeneous and many more samples would have to be taken in order to reduce the standard error of the mean to a reasonable figure. This was physically impossible with the sampling apparatus being used. A careful consideration of the objectives of the project together with a review of the techniques employed pointed the way to the most likely solution to the problem. The basic objective was to determine whether or not the treatments in question reduced the plankton population sufficiently to interfere with the development of fish and other higher organisms that depend upon plankton for food. Any differential effect upon nannoplankton might be of great biological interest but would be of only secondary importance to the present project. Placing major importance upon total volume relationships and relative abundance of the larger taxonomic groups of mesoplankton would speed up the counting process so that many more samples could be counted. At the same time the microbiologist modified his sampling apparatus in such a way that it would be possible to take many more samples from a given pond and several samples from the same spot if desired. Moreover it appeared that a relatively large temporary reduction in population of plankton might be possible without serious effect upon the fish population. Thus a test that would detect a twenty-five percent level of significance would probably be sufficiently sensitive. Due to the lack of readily available information as to the power of the t-test, it was necessary to consult a mathematical statistician in order to determine the number of replications necessary to meet these specifications.

Another example of the value of apparently elementary biometrical advice is to be found in the selection of the best dosage series to test in any particular experiment. General advice such as using a geometrical series rather than an arithmetical series is of value

only when it is applied to a specific case. Thus in an experiment designed to test the effectiveness of a certain insecticide against fly larvae, the investigator was physically limited to twenty or thirty plots, though it would be possible to repeat the entire experiment before a decision had to be reached. The practical range of dosages would extend to about 2000 units but no estimate of the standard deviation was available. The biologist favored a series of 0, 200, 400, 500, 750, 1000, 1250, 1500, and 2000 units. This would restrict him to three replications in his initial experiment. The advice of the biometrician in this case was to reduce the number of treatments by using a geometrical series and to increase the number of replicates accordingly. thus providing a better estimate of the standard deviation in the initial experiment and at the same time determining fairly closely the effective range. Then the second experiment could be set up to determine more accurately the slope of the curve in the effective range, and the number of replicates necessary to produce significant figures could be estimated. The investigator still felt that a jump in dosage from 800 to 1600 units was too great, so a compromise series of 0, 200, 400, 800, 1200, and 1600 units was agreed upon.

Another way in which a biometrician may serve a laboratory in which scientists of varied training are working on the same general project is by suggesting that determinations and observations be made with approximately equal accuracy by all concerned. If one scientist is limited to rather low accuracy, the others should not slow down the progress of the entire project by attempting to improve their own accuracy beyond the limits of their colleagues. Thus if an insecticide is being evaluated by both chemical and biological methods in the same laboratory, the chemical evaluation is apt to be much more accurate than the biological. Because chemists are generally trained to expect greater accuracy than are biologists it sometimes happens that the chemist delays the program attempting to reduce his coefficient of variation to one or two percent when the five percent that he has already attained is far better than the ten to fifteen per-

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It is not necessary to restrict examples to any one field of endeavor. Agricultural entomology is particularly rich in excellent examples. In numerous cases such as in the control of turnip aphids, the initial population may be so high that casual inspection is enough to prove that the economic value of the crop is being destroyed. Moreover the only control of any practical concern is one which reduces the population to the point where both grower and buyer are satisfied on the basis of casual observation. In such a case, especially with insects as difficult to count as aphids, there may be no justification for taking the time necessary for actual counts. Obviously no mathematical analysis is possible, vet carefully planned experiments are essential to make sure that it was the treatment rather than natural causes that produced the control. Again with insects difficult to count, such as leafhopper nymphs, the biometrician may be able to suggest a valid shortcut to counts, such as estimating the mean by the proportion of uninfested leaves in case of a Poisson distribution.

These few examples, though not exhaustive, should indicate some of the many ways in which a biometrician can serve applied biology. In time of war, when important decisions must be made rapidly, it is not always possible to use the one percent level of significance. As an Army officer recently returned from the battle field was heard to say, "If I have more than the necessary material at the required time and place then I am a hero, though possibly an expensive hero. If it develops that the material was not needed, then I will not be criticized too severely. But if it is needed and I do not have it there, then I am a failure and the battle may be lost." Applying this philosophy to wartime biological research, it is not

so important that the biologist detect small differences between similar treatments. If he rejects one treatment in favor of a slightly more expensive treatment or a slightly less effective one no one will criticize him too severely. But if he is so busy determining with great accuracy the relative efficiency of two similar treatments that he completely overlooks a vastly superior treatment, then he is a failure. The man who discovers the workable procedure, even though it may not be the most efficient, is the hero at the present time. Recognizing this state of affairs as being inevitable in time of war and of considerable importance, particularly to commercial concerns, in time of peace, the biometrician must strive to fit his advice to this situation. The statistical advice most highly prized by the biologist is that which enables him to know that a large apparent difference in effectiveness is due to the treatment rather than to some overlooked or uncontrolled factor.

In order to give this type of advice the biometrician must be thoroughly trained in the principles of statistical reasoning which is essentially the logic of experimentation. Familiarity with highly involved experimental designs and methods of analysis may be a liability unless the biometrician is able to lay them aside and revert to the basic principles which are often called "common sense." All experimental scientists should be familiar with these principles, but many of them are not. A man may be an authority on the biology and taxonomy of certain biological groups and yet shun anything mathematical. He may have invaluable knowledge of and ability to develop biological or mechanical methods and techniques without appreciating the value of simple replication in his tests. He may even have brilliant ideas regarding radically new and different treatments to try out without knowing just how to test them properly. The research director should be and usually is familiar with the logic of experimentation but in even a medium-sized laboratory he is often too busy with objectives, policies, and administrative work to be able to check the details of design and analysis in all of the projects. Therefore, a biometrician who can actually work some on each project can be of incalculable value to a research group that is trying to produce a large volume of reliable results in a short time. The minute distinctions between similar results may be missing but the general conclusions should be reliable.

DEPARTMENT OF BIOSTATISTICS AT THE SCHOOL OF HYGIENE AND PUBLIC HEALTH, THE JOHNS HOPKINS UNIVERSITY

The School of Hygiene and Public Health offers no undergraduate work and its students fall into two rather distinct classes. First, medical graduates training for administrative public health positions such as that of health officer, hospital administrator, epidemiologist, and so forth; and second, graduate students who may or may not be medical men, training as specialists in one of the sciences such as biochemistry, parasitology, bacteriology, or biostatistics. The Department of Biostatistics gives some instruction to practically all of these students and extensive training to a small number accepted as students in the Department. It therefore has a rather varied teaching problem since its students include both mature men with considerable professional experience but negligible mathematical background, and science degree students some of whom have quite rich undergraduate training in mathematics. An additional source of both interest and complexity in the teaching problem is the international character of the School. Of the approximately 1300 past public health graduates of the School, about 40 percent have come from 51 different countries outside the United States.

The routine teaching responsibility of the Department is to present a basic course in statistics to the public health students and a similar course to the first-year students in the School of Medicine. In the prewar years there were about 70 students accepted in a vear as candidates for the Master of Public Health degree, and the first-year medical class was slightly larger. With all the varied experience of these students, they are apt to have one thing in common, and that is a very limited background in quantitative method. The object of the required course for both the public health students and the medical students is therefore primarily to give them a concept of some of the issues involved in quantitative reasoning in science, and at the same time to give instruction in those statistical techniques which they most frequently need.

The course for the public health students is presented in 24 hours of lecture and 72 hours of laboratory work. A liberal number of laboratory instructors is provided and the students debate the issues of the problem with them and with each other. The variation in past training and experience of the group thus ceases to be a problem and becomes an asset since the students can proceed at their own pace and have the advantage of contact with individuals having varied scientific backgrounds.

The course for the medical students is about half as long and deals to a large extent with the principles involved in rate reasoning, since the drawing of sound conclusions from simple ratios is by far the most important quantitative problem that the medical man meets in either laboratory or clinical experience. The placing of the course in the first year has been done advisedly, in spite of the limited medical knowledge of the students at that time, since in the clinical years the attention is too much focused on the individual case for effective presentation of statistical concepts.

Students studying to be specialists in biostatistics may become candidates for the Doctor of Science in Hygiene degree. Candidates are required to have for admission mathematics through the calculus, and the basic pre-medical and medical sciences. Work for the degree involves three years of graduate study and the completion of a dissertation.

There are several formal courses open to such students. The course previously mentioned is the first of three courses in the elements of statistical methodology. The subject matter is presented in seminars, lectures, and laboratory work, a large proportion of cent of the biological assay. A specific example of this sort is hardly necessary but it should be the duty of the biometrician in such a laboratory to keep track of the approximate accuracy being obtained by the different techniques involved in a given project and to attempt to keep them balanced so that all phases of the research can advance together.

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so important that the biologist detect small differences between similar treatments. If he rejects one treatment in favor of a slightly more expensive treatment or a slightly less effective one no one will criticize him too severely. But if he is so busy determining with great accuracy the relative efficiency of two similar treatments that he completely overlooks a vastly superior treatment, then he is a failure. The man who discovers the workable procedure, even though it may not be the most efficient, is the hero at the present time. Recognizing this state of affairs as being inevitable in time of war and of considerable importance, particularly to commercial concerns, in time of peace, the biometrician must strive to fit his advice to this situation. The statistical advice most highly prized by the biologist is that which enables him to know that a large apparent difference in effectiveness is due to the treatment rather than to some overlooked or uncontrolled factor.

In order to give this type of advice the biometrician must be thoroughly trained in the principles of statistical reasoning which is essentially the logic of experimentation. Familiarity with highly involved experimental designs and methods of analysis may be a liability unless the biometrician is able to lay them aside and revert to the basic principles which are often called "common sense." All experimental scientists should be familiar with these principles, but many of them are not. A man may be an authority on the biology and taxonomy of certain biological groups and yet shun anything mathematical. He may have invaluable knowledge of and ability to develop biological or mechanical methods and techniques without appreciating the value of simple replication in his tests. He may even have brilliant ideas regarding radically new and different treatments to try out without knowing just how to test them properly. The research director should be and usually is familiar with the logic of experimentation but in even a medium-sized laboratory he is often too busy with objectives, policies, and administrative work to be able to check the details of design and analysis in all of the projects. Therefore, a biometrician who can actually work some on

each project can be of incalculable value to a research group that is trying to produce a large volume of reliable results in a short time. The minute distinctions between similar results may be missing but the general conclusions should be reliable.

DEPARTMENT OF BIOSTATISTICS AT THE SCHOOL OF HYGIENE AND PUBLIC HEALTH, THE JOHNS HOPKINS UNIVERSITY

The School of Hygiene and Public Health offers no undergraduate work and its students fall into two rather distinct classes. First, medical graduates training for administrative public health positions such as that of health officer, hospital administrator, epidemiologist, and so forth; and second, graduate students who may or may not be medical men, training as specialists in one of the sciences such as biochemistry, parasitology, bacteriology, or biostatistics. The Department of Biostatistics gives some instruction to practically all of these students and extensive training to a small number accepted as students in the Department. It therefore has a rather varied teaching problem since its students include both mature men with considerable professional experience but negligible mathematical background, and science degree students some of whom have quite rich undergraduate training in mathematics. An additional source of both interest and complexity in the teaching problem is the international character of the School. Of the approximately 1300 past public health graduates of the School, about 40 percent have come from 51 different countries outside the United States.

The routine teaching responsibility of the Department is to present a basic course in statistics to the public health students and a similar course to the first-year students in the School of Medicine. In the prewar years there were about 70 students accepted in a year as candidates for the Master of Public Health degree, and the first-year medical class was slightly larger. With all the varied experience of these students, they are apt to have one thing in common, and that is a very limited background in quantitative method. The object of the required course for both the public health students and the medical students is therefore primarily to give them a concept of some of the issues

involved in quantitative reasoning in science, and at the same time to give instruction in those statistical techniques which they most frequently need.

The course for the public health students is presented in 24 hours of lecture and 72 hours of laboratory work. A liberal number of laboratory instructors is provided and the students debate the issues of the problem with them and with each other. The variation in past training and experience of the group thus ceases to be a problem and becomes an asset since the students can proceed at their own pace and have the advantage of contact with individuals having varied scientific backgrounds.

The course for the medical students is about half as long and deals to a large extent with the principles involved in rate reasoning, since the drawing of sound conclusions from simple ratios is by far the most important quantitative problem that the medical man meets in either laboratory or clinical experience. The placing of the course in the first year has been done advisedly, in spite of the limited medical knowledge of the students at that time, since in the clinical years the attention is too much focused on the individual case for effective presentation of statistical concepts.

Students studying to be specialists in biostatistics may become candidates for the Doctor of Science in Hygiene degree. Candidates are required to have for admission mathematics through the calculus, and the basic pre-medical and medical sciences. Work for the degree involves three years of graduate study and the completion of a dissertation.

There are several formal courses open to such students. The course previously mentioned is the first of three courses in the elements of statistical methodology. The subject matter is presented in seminars, lectures, and laboratory work, a large proportion of

the time being allotted to the last. Other formal courses in theory and application are offered within the period of residence of a candidate although not all in one year. These include courses in probability theory, analysis of small samples, theory of life table construction, demography, and the quantitative treatment of laboratory procedures. are also applied courses in vital statistics and hospital statistics. One rather unusual teaching experiment is the presentation of a course jointly with the Department of Epidemiology. This is a seminar and laboratory course in which the subject matter of the field is presented by the epidemiology department and the methodology for treating the material is given by the biostatistics department. This approach to the theory through the problems creating the need for it is a combined stimulus toward the development of new methodology to meet the requirements of the applied field.

The research program of the Department is varied and includes work in both mathematical statistics and applied problems in many phases of quantitative biology. Besides the investigations initiated in this Department, quantitative medical problems under investigation in other parts of the environment are at times handled by research teams which include members of the biostatistics staff. The staff also does a large amount of consultant work around problems arising in other departments in the School of Hygiene, in the School of Medicine, and in outside research

units. The breadth of the cooperative research problem has strengthened in the minds of the staff the concept that statistics, in its relation to the advancement of science, should not be concerned solely with the subject of sampling variation, but rather with all aspects of sound interpretation for man's quantitative observations; that is, that statistics is applied mathematics in its broadest sense. Under this idea, the statistician should be as much concerned with the creation of the hypothesis under which the observations are to be tested as he is with methods of testing the observations under the hypothesis.

The opportunities open to persons trained in biostatistics greatly exceed the supply of such people. The demand for such specialists has markedly increased in recent years because of the movement to put courses in quantitative method into the medical curriculum and through the growing appreciation of the need for sound statistical analysis in biological research.

The Committee in charge of awarding degrees of Doctor of Science in Biostatistics is the Advisory Board of the School of Hygiene consisting of the full professors in the School. The staff of the Department of Biostatistics consists of Lowell J. Reed, Professor and Head of the Department; Margaret Merrell, Associate Professor; A. W. Hedrich and W. T. Fales, Lecturers; and E. L. Crosby, G. F. Badger, and M. Pascua, Assistant Professors.

NEWS AND NOTES

A symposium on Mathematical Statistics and Probability was held at the Berkeley campus of the University of California, August 13-18 inclusive. Speakers and chairmen included G. P. Adams, E. M. Beesley, Joseph Berkson, B. A. Bernstein, A. H. Copeland, G. B. Dantzic, P. H. Daus, J. L. Doob, F. W. Dresch, G. C. Evans, Harold Hotelling, P. L. Hsu, Victor P. Lenzen, J. H. McDonald, A. H. Mobray, J. Neyman, G. Polya, Hans Reichenbach, A. C. Schaeffer, J. D. Tamarkin, Morgan Ward, Jacob

WOLFOWITZ.

A. L. BACHRACH, with the Glaxo Laboratories Ltd., Greenford, Middlesex, writes "We are about, in this country, to found a new group of the Society of Public Analysts and Other Analytical Chemists, to be known as "The Biological Methods Group," which will devote its attention to the technique of quantitative tests involving the use of whole animals or parts thereof. Naturally, consideration of statistical methods will play a large part in the work of this section, but it is in-

tended to consider other matters than the design and evaluation of experiments. Such problems as the conditions for breeding and feeding satisfactory animal stock would fall within the scope of the section." The first meeting of this group is to be held in October . . . EDWIN J. de BEER, assistant director, The Wellcome Research Laboratories, has written a most helpful letter full of comments and suggestions regarding the Bulletin. We want to assure you and other readers that the only way we can continue "Queries" section and make it helpful is for you to send your questions to G. W. SNEDECOR. We agree, "there are many problems worthy of discussion by authorities and the airing of such discussions is very helpful to the rank and file as well as to the experts." What problems do you want discussed? . . .

ROY A. CHAPMAN. Silviculturist in charge,

Hitchiti Experimental Forest, Round Oaks, Georgia, has expressed his belief that the Queries section will be particularly valuable. We will try to get statisticians to write articles on the subjects you and others are suggesting . . J. FINNEY, Rothamsted, has been elected to a Lectureship in the Design and Analysis of Scientific Experiment at Oxford. He will go there in October . . . LT. WILLIAM M. UPHOLT left the South Carolina Experiment Station in 1942. He spent two years with the California Spray Chemical Corporation doing field research and service work for them in Florida agriculture. In July 1944 he took military leave from California Spray to accept a commission in the U. S. Public Health Service. His assignment at Carter Memorial Laboratory has kept him doing entomological research . . . RICHARD H. BLYTHE, JR., Forest Economist with the Forest Service, U.S.D.A. sent in his comments and suggestions for the Bulletin ... SEWALL WRIGHT, Department of Zoology, the University of Chicago, has been dealing with "path coefficients" until his paths are numerous. His comments are "I am on six other Editorial Boards." . . . HORACE NOR-

TON, Weather Bureau, writes, "I have been

working on quite the most vexing problem I have ever tackled, and that is the more curious

because it is a problem of curve fitting (distribution) which of course has been well understood and completely solved in principle since 1922." . . . On July 2nd and 3rd, C. F. SARLE and C. E. LAMOUREUX of the United States Weather Bureau conferred with J. W. HARRELSON, Chancellor; I. O. SCHAUB, Dean of the School of Agriculture and Forestry; L. D. BAVER, Director of the Agricultural Experiment Station; J. H. LAMPE, Dean of the Engineering School; and other members of the faculty of the North Carolina State College on the subject of the expanded program of the U.S. Weather Bureau Station at Raleigh with particular reference to the use of machine tabulation and modern statistical methods as applied to climatological data. Mr. Lamoureux will be the new Section Director at Raleigh. For the last three years he has been assigned to the Army Air Forces Weather Service where he has been preparing numerous climatological reports with military applications. Climatological data will be made available in usable form for the benefit of the agricultural, engineering, industrial and commercial interests of the State . . . C. M. KINCAID now doing graduate work at Iowa State College, expects to return to the Virginia Polytechnic Institute, Blacksburg, by the first of September in time to extend a welcome and birthday greetings (September 19) to D. B. DeLURY. There is considerable interest about the Ames Statistical Laboratory in the estimation of variance components . . . A. E. BRANDT is back in Vienna, Virginia where he is busy getting acquainted with his family and relaxing after two years of duty overseas . . . JOSEPH F. PECHANEC, Forest Ecologist, Dubois, Idaho, is being transferred to Portland, Oregon, to be in charge of Range Research work at the Pacific Northwest Forest and Range Experiment Station . . . DAVID B. DUNCAN of the Royal Australian Air Force has recently arrived at Iowa State College from Sydney, Australia, to take advanced work in statistics. Before the war. Mr. Duncan was a lecturer in Agricultural Biometry at the University of Australia . . . W. G. COCHRAN has just returned from Europe.

QUERY During a study of the isolating mechanisms between two species of Drosophila, males of *D. persimilis* were first confined for several days with females of their own species (pro-conditioned) then given access to an (originally) equal number of females of their own species and of *D. pseudoobscura*. In a second experiment the males were first confined with females of *D. pseudoobscura* (counter-conditioned) and then given the multiple choice. The resulting numbers of dissected females carrying and not carrying sperm were as follows:

$$18 + x 14 - x$$
 $41 - x 6 + x$
 $5 - x 32 + x$ $20 + x 32 - x$

it is clear that the hpyothesis to be tested is

$$\frac{(18+x)(32+x)}{(5-x)(14-x)} = \frac{(41-x)(32-x)}{(20+x)(6+x)}$$

If these two hypothetical interactions are equal, then x can be determined by solving the equation,

$$(18 + x) (32 + x) (20 + x) (6 + x) =$$

 $(5 - x) (14 - x) (41 - x) (32 - x)$

If you are familiar with Horner's method of calculating the approximate real roots of

		Pro- conditioned		Counter- conditioned	
	Insemi- nated	Not	Insemi- nated	Not	
Persimilis	18	14	41	6	
Pseudoobscura	- 5	32	20	32	

What I would like to determine is the probability that the pro-conditioned and counter-conditioned samples are not merely samples of the same population.

ANSWER The interaction in the 2 x 2 table of pro-conditioned insects is

$$\frac{18/5}{14/32} = \frac{(18)(32)}{(5)(14)} = 8.23,$$

while that in the counter-conditioned is $\frac{(41)(32)}{(20)(6)} = 10.93.$

I think you wish to know if these interactions differ significantly; that is, if the second order interaction.

10.93/8.23 = 1.33,

differs significantly from unity.

M. S. Bartlett gave the solution to this problem in the Supplement to the Journal of the Royal Statistical Society, Vol. 2, page 248, in 1935. The 2 x 2 x 2 table has but a single degree of freedom and a single deviation, x, by which each observed value departs, positively or negatively, from the corresponding expected value. If x is combined with the observed numbers as follows,

such an equation, the solution is not difficult. If not, and if you have a calculating machine available, you can often locate the root by trial without much labor. For example, try x = 0: the left member is 69,120 while the right is 91,840, a difference of -22,720. Next try x = 1: left is 92,169; right, 64,480; difference, 27,689. The fact that the sign has changed indicates a root between 0 and 1, while the relative sizes of the differences, sign ignored, point to a root almost half way between, but slightly nearer 0. Hence, try 0.4 and 0.5. For the former, left is now 77,835; right. 80,262; difference, -2,427. For the latter, left is 80,117; right, 77,502; difference, 2,615. Clearly, the root is almost half way between 0.4 and 0.5, indicating x = 0.45 approxi-This leads to the set of expected mately. numbers,

Hence,

$$\chi^{9} = (0.45)^{2} \left(\frac{1}{18.45} + \frac{1}{13.55} + \dots + \frac{1}{31.55} \right)$$

= (0.2025) (0.6389) = 0.13, d. f.=1, P=0.72

If x were greater than 0.5, it would be proper to deduct Yates' correction before squaring.

The case of the 2ⁿ x R table has been discussed by H. W. Norton in the Journal of the American Statistical Association, Vol. 40, page 251, June 1945.

G. W. SNEDECOR.

QUERY In the first problem under "Queries" you gave six as the degrees of freedom for cages receiving the same treatment. I thought degrees of freedom were always one less than something, yet six is merely half the number of cages.

ANSWER The phrase, degrees of freedom, refers to the number of independent values of

the variate after certain restrictions have been imposed. The degrees of freedom are often one less than the number of observations, n, because a single restriction is commonly set up—that deviations from mean shall be the variate used. Since the sum of such deviates is zero, onlyn — 1 of them are independent.

This rule is applied six times in calculating the sum of squares for cages receiving the same treatment, once in each pair of cages treated alike. Hence one is deducted from the n=2 cages in each of the six pairs.

Another way to think of it is this: Experimental error is based on the differences between means of egg production in the several pairs treated alike, and there are six independent such differences.

G. W. SNEDECOR.

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Material for the BULLETIN should be addressed to the Chairman of the Editorial Committee, Institute of Statistics, North Carolina State College, Raleigh, N. C., material for Queries should go to "Queries", Statistical Laboratory, Iowa State College, Ames, Iowa, or to any member of the committee.